

### AMENDMENTS TO THE CLAIMS

Please amend claims 1, 9, 20, 42, 64, 66 and 70, as set forth in the following listing of claims, which will replace all prior versions, and listings, of claims in the present application.

#### Listing of Claims

1. (Currently Amended) A method of injecting a liquid volume into a microchannel, comprising the steps of:
  - providing a microchannel filled with a first fluid, wherein the microchannel includes a fluid interface port formed in the sidewall of the microchannel, the fluid interface port having a depth equal to a thickness of the sidewall and a diameter that is significantly larger than the depth, wherein the first fluid forms virtual wall comprising a meniscus of the first fluid formed at the fluid interface port that is co-planar with the sidewall, such that none of the first fluid in the microchannel enters the fluid interface port when the virtual wall is formed;
  - forming a droplet from the liquid; and
  - directing the droplet to the virtual wall formed by the first fluid in a fluid interface port formed in a sidewall of the microchannel, such that the droplet of the liquid traverses the virtual wall.
2. (Withdrawn) The method of claim 1, further comprising the step of charging the droplet after forming the droplet.
3. (Withdrawn) The method of claim 2, wherein the step of directing comprises establishing an electrostatic field to guide the charged droplet to the virtual wall.
4. (Withdrawn) The method of claim 2, further comprising the step of charging the microchannel to attract the charged droplet.
5. (Withdrawn) The method of claim 4, wherein the step of charging the microchannel comprises the step of connecting the microchannel to an electrode and a channel charging circuit.
6. (Withdrawn) The method of claim 4, further comprising the step of charging a neighboring microchannel.

7. (Withdrawn) The method of claim 6, further comprising the step of charging the neighboring microchannel with a charge opposite that of the microchannel.
8. (Withdrawn) The method of claim 2, wherein the step of directing comprises the step of charging a target electrode positioned adjacent to the virtual wall to guide the droplet to the virtual wall.
9. (Currently Amended) A method of filling a microchannel with a liquid, comprising:  
forming one or more droplets of the liquid; and  
directing the droplet towards a fluid interface port formed in a side wall of the microchannel which communicates with an interior portion thereof such that the droplet traverses the fluid interface port and enters the interior, wherein the fluid interface port has a depth equal to a thickness of the sidewall, and a diameter that is significantly larger than the depth, and  
directing droplets through the fluid interface port until the microchannel is filled and a virtual wall fluid interface port comprising a meniscus of the first fluid is formed in the fluid interface port, such that the meniscus is co-planar with the side wall, wherein none of the liquid in the microchannel enters the fluid interface port when the microchannel is filled with the liquid and the virtual wall is formed.
10. (Original) The method of claim 9, wherein the fluid interface port is a filling aperture.
11. (Original) The method of claim 9, further comprising the step of forming a pressure barrier in the microchannel to force the liquid in a first direction.
12. (Original) The method of claim 11, further comprising the step of providing a stopper hole in the sidewall of the microchannel to form the pressure barrier.
13. (Original) The method of claim 11, further comprising the step of disposing a hydrophobic patch within the interior of the microchannel to form the pressure barrier.
14. (Original) The method of claim 9, further comprising the step of providing a vent hole in the sidewall of the microchannel for allowing air to escape therefrom.
15. (Canceled)

16. (Original) The method of claim 9, further comprising the step of closing the fluid interface port after the microchannel is filled with the liquid.

17. (Original) The method of claim 16, wherein the step of closing comprises:  
forming drops of an encapsulant; and  
dispensing the drops of the encapsulant onto the fluid interface port to close the fluid interface port.

18. (Original) The method of claim 16, wherein the step of closing comprises the step of closing the fluid interface port with a covering layer.

19. (Original) The method of claim 18, further comprising the step of adhering the covering layer to the microchannel on top of the fluid interface port.

20. (Currently Amended) A method of filling a microchannel with a plurality of liquids, comprising:

providing a microchannel comprising a side wall encompassing a hollow interior having a first diameter of between about 25  $\mu\text{m}$  and about 100  $\mu\text{m}$ , a first filling aperture formed in the side wall to provide access to the interior, wherein the first filling aperture has a second diameter that is substantially equal to the first diameter and a depth that is equal to a thickness of the sidewall and is significantly smaller than the first diameter, the microchannel further including a second filling aperture formed in the side wall to provide access to the interior, wherein the second filling aperture has a third diameter that is substantially equal to the first diameter;

forming one or more droplets of the first liquid;

directing a droplet of the first liquid towards the first filling aperture in the side wall such that the droplet traverses the filling aperture and enters the interior;

forming one or more droplets of a second liquid;

directing a droplet of the second liquid towards the second filling aperture in the side wall such that the droplet of the second liquid traverses the second filling aperture and enters the interior, wherein none of the first liquid fills the first filling aperture ~~when~~ and none of the second liquid fills the second filling aperture when the microchannel is filled with the first liquid and the second liquid and a virtual wall fluid meniscus is formed in each of the first filling aperture and the second filling aperture.

21. (Original) The method of claim 20, wherein the first liquid and the second liquid are immiscible with each other and do not mix when disposed in the interior of the microchannel.

22. (Withdrawn) A method of performing a liquid to liquid extraction technique, comprising the steps of:

providing a microchannel having a side wall, an interior chamber, a fluid interface port, and a target liquid disposed in the interior chamber to form a virtual wall in the fluid interface port; and

injecting a source liquid through the virtual wall and into the target liquid to extract a substance from the source liquid.

23. (Withdrawn) The method of claim 22, further comprising the step of collecting the target liquid after the target liquid extracts the substance from the source liquid.

24. (Withdrawn) The method of claim 22, further comprising the step of separating the source liquid from the target liquid after the target liquid extracts the substance from the source liquid.

25. (Withdrawn) The method of claim 22, wherein the source liquid is immiscible with the target liquid.

26. (Withdrawn) The method of claim 22, wherein the source liquid comprises an organic solvent and the substance comprises a water-soluble substance.

27. (Withdrawn) The method of claim 22, wherein the target liquid comprises an aqueous solution.

28. (Withdrawn) The method of claim 22, further comprising the step of ejecting the source liquid from the interior through the virtual wall.

29. (Withdrawn) A method of processing a liquid, comprising:

providing a microfluidic system comprising a substrate and a microchannel for housing a first fluid formed in the substrate, said microchannel comprising a side wall, an interior, and a plurality of fluid interface ports such that that the first fluid therein forms a virtual wall in each of said plurality of fluid interface ports;

injecting a second liquid into the first liquid in the microchannel through said virtual walls; and

performing a chemical manipulation on the second liquid and the first liquid to form a reacted fluid.

30. (Withdrawn) The method of claim 29, further comprising the step of ejecting the reacted fluid through the virtual wall.

31. (Withdrawn) The method of claim 30, wherein the step of ejecting the reacted fluid comprises the step of establishing an electric field to attract the reacted fluid through the virtual wall.

32. (Withdrawn) The method of claim 31, wherein the step of establishing an electric field comprises the steps of

disposing a first electrode in the microchannel and a second electrode adjacent to one of said virtual walls outside of the microchannel, and

applying a potential difference between the first electrode and the second electrode to form the electric field.

33. (Withdrawn) The method of claim 29, wherein the step of ejecting the reacted fluid comprises the step of applying a pressure pulse to the reacted fluid.

34. (Withdrawn) The method of claim 29, wherein the step of ejecting the reacted fluid comprises the step of applying a gas pressure pulse to the reacted fluid.

35. (Withdrawn) The method of claim 34, wherein the gas pressure pulse is generated by a third virtual wall in the microchannel disposed substantially coaxially with the second virtual wall.

36. (Withdrawn) The method of claim 29, wherein the step of ejecting the reacted fluid comprises the step of applying heat to the reacted fluid.

37. (Withdrawn) The method of claim 29, further comprising the step of detecting the reacted fluid.

38. (Withdrawn) The method of claim 37, wherein the step of detecting comprises the step of focusing a light beam with an optical element, said light beam impinging upon the fluid in the microchannel through the virtual wall.

39. (Withdrawn) The method of claim 29, further comprising the step of injecting a third liquid into the microchannel through one of said virtual walls.

40. (Withdrawn) The method of claim 29, wherein the chemical manipulation comprises a reaction on an analyte.

41. (Withdrawn) The method of claim 40, wherein the chemical manipulation comprises a labeling operation.

42. (Currently Amended) A method of introducing a second liquid into a microchannel filled with a first fluid, said method comprising:

forming a droplet from the second liquid,

introducing said droplet through a virtual wall formed by the first fluid in a fluid interface port formed in a sidewall of the microchannel, wherein the fluid interface port has a depth equal to a thickness of the sidewall, a diameter that is significantly larger than the depth, and a dead volume of less than about one picoliter, wherein the virtual wall has a meniscus surface that is substantially co-planar with the side wall in which the virtual wall is formed such that none of the first fluid from the microchannel enters the fluid interface port when the virtual wall is formed.

43. (Original) The method of claim 42, wherein the microchannel further comprises a second fluid interface port, formed in the sidewall, disposed opposite to the fluid interface port and arranged coaxially therewith.

44. (Original) The method of claim 42, further comprising the step of employing the virtual wall as an optical window for optically detecting one of the fluids in the microchannel.

45. (Original) The method of claim 44, further comprising the step of optically detecting the fluid in the microchannel through the virtual wall formed in the fluid interface port.

46. (Canceled)

47. (Original) The method of claim 42, wherein the fluid interface port has zero dead volume.

48. (Original) The method of claim 42, wherein the step of introducing the droplet comprises the step of introducing the droplet into the virtual wall at a speed and a direction such that the droplet traverses the virtual wall.

49. (Withdrawn) The method of claim 42, further comprising the step of charging the droplet to form a charged droplet.

50. (Withdrawn) The method of claim 49, further comprising the step of generating an electric field to guide the charged droplet towards the virtual wall.

51. (Withdrawn) The method of claim 49, further comprising the step of deflecting the charged droplet with at least one electrically controlled deflection plate.

52. (Withdrawn) The method of claim 42, further comprising a plurality of fluid interface ports formed in said sidewall, the method further comprising the steps of  
charging the droplet to form a charged droplet,  
charging the microchannel, and  
selecting one of said plurality of fluid interface ports to introduce the charged droplet.

53. (Withdrawn) The method of claim 42, further comprising a plurality of microchannels having one or more fluid interface ports, the method further comprising the steps of  
charging the droplet to form a charged droplet,  
charging one or more of the plurality of microchannels, and  
selecting at least one of said channels to introduce the charged droplet.

54. (Withdrawn) The method of claim 53, further comprising the step of selecting at least one of said fluid interface ports of one of said plurality of microchannels to introduce the charged droplet.

55. (Original) The method of claim 42, wherein the droplet is immiscible with the first liquid.

56. (Previously Presented) The method of claim 42, further comprising the step of moving the second liquid in the microchannel.

57. (Original) The method of claim 56, wherein the step of moving the fluid comprises applying an electric field to the first fluid to move the fluid within the microchannel.

58. (Original) The method of claim 42, wherein the droplet has a diameter that is smaller than the fluid interface port.

59. (Withdrawn) The method of claim 42, further comprising the steps of providing an array of fluid interface ports and forming the droplet to have a diameter that is substantially larger than one of said array of fluid interface ports.

60. (Withdrawn) The method of claim 59, further comprising the step of introducing the droplet to said array of fluid interface ports.

61. (Original) The method of claim 42, further comprising the step of forming the droplet on a droplet carrying element.

62. (Original) The method of claim 61, wherein the step of introducing comprises the step of applying the droplet to the virtual wall with the droplet carrying element.

63. (Original) The method of claim 42, further comprising the steps of:  
forming a second droplet from a third liquid; and  
injecting the second droplet through a second virtual wall formed in a second fluid interface port formed in the microchannel.

64. (Currently Amended) A method of injecting a second liquid into a microchannel filled with a first fluid, said method comprising:  
forming a droplet from the second liquid,  
introducing said droplet through a virtual wall formed by the first fluid in a fluid interface port formed in a sidewall of the microchannel, said fluid interface port having a diameter between about 25  $\mu\text{m}$  and about 100  $\mu\text{m}$ , a depth that is equal to a thickness of the sidewall and is significantly smaller than the first diameter, and a dead volume of less than about one picoliter, wherein the virtual wall has a meniscus surface that is substantially coplanar with the side wall in which the virtual wall is formed, such that none of the first fluid in the microchannel enters the fluid interface port when the virtual wall is formed.

65. (Canceled)



66. (Currently Amended) A method of injecting a second liquid into a microchannel filled with a first fluid, said method comprising:

forming a droplet from the second liquid,

introducing said droplet through a virtual wall formed by the first fluid in a first fluid interface port formed in a sidewall of the microchannel, wherein the fluid interface port has a depth equal to a thickness of the sidewall, a diameter that is significantly larger than the depth, and, wherein the virtual wall has a meniscus surface that is substantially co-planar with the side wall in which the virtual wall is formed, such that none of the first fluid in the microchannel enters the fluid interface port when the virtual wall is formed.

67. (Withdrawn) A method of separating a sample into different components according to size, comprising

providing a microchannel having an interior bounded by a side wall, a fluid interface port formed in the side wall of the microchannel to provide access to the interior of the microchannel, and a fluid washing medium disposed in the interior of the microchannel and forming a virtual wall at the fluid interface port,

inducing a flow in the washing medium,

forming a droplet of the sample,

injecting the droplet through the virtual wall into the flowing washing medium, such that the components of the sample separate by diffusion into the washing medium at different diffusion rates and wherein the diffusion rate of a component is determined by particle size.

68. (Withdrawn) The method of claim 67, further comprising the step of collecting the diffused components.

69. (Withdrawn) The method of claim 67, wherein the sample comprises DNA and contaminants and the step of injecting the droplet of the sample into the washing medium purifies the DNA by separating the contaminants from the DNA.

70. (Currently Amended) A method of injecting a liquid volume into a microchannel, filled with a first fluid comprising the steps of:

forming a droplet from the liquid; and

directing the droplet through a virtual wall formed by the first fluid in a fluid interface port formed in a sidewall of the microchannel, wherein none of the first fluid in the

microchannel enters the fluid interface port when the microchannel is filled with the first fluid and the virtual wall is formed,

wherein the virtual wall has a meniscus surface that is substantially co-planar with the side wall in which the virtual wall is formed and the fluid interface port has a depth equal to a thickness of the sidewall and a diameter that is significantly larger than the depth.